Kidney International, Vol. 45 (1994), pp. 345–351

Loss of glomerular function and tubulointerstitial fibrosis: Cause or effect?

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The relentless progression of many renal diseases to end-stage renal failure after an apparently transient initial insult remains an enigma that continues to fascinate nephrologists. The "hyperfiltration" hypothesis based on the rat remnant kidney model of nephron ablation provided much of the intellectual stimulus to the study of glomerular injury and scarring in the progression of renal disease [1]. While this may have some relevance to human renal disease progression [2], the early work of Risdon et al [3], Schainuck et al [4] and Bohle et al [5] has made it clear that, perhaps paradoxically, it is the degree of tubulointerstitial pathology that correlates most closely with declining renal function even in classical "glomerular" diseases. Belated recognition has now given rise to a new interest in the pathological processes occurring in the tubulointerstitial compartment of the diseased kidney. In this paper, we review briefly the histopathological evidence for the involvement of the tubulointerstitium in progressive renal disease and discuss potential mechanisms for tubulointerstitial injury. We also discuss pathways via which tubulointerstitial disease may lead to loss of glomerular function and propose a unifying hypothesis which links the two, taking into account much of the known experimental evidence at present.

Tubulointerstitial changes correlate with renal disease progression

In 1968, while attempting to find a histological correlate for functional renal impairment in patients with chronic glomerulonephritis, Risdon and his colleagues arrived at what appeared to be an anomalous finding: a positive correlation between tubular atrophy and a decline in glomerular filtration rate (GFR), but no significant correlation between glomerular damage and GFR [3]. Soon afterwards, Schainuck and Striker et al extended this observation with detailed studies of renal function in patients with a variety of nephropathies [4, 6]. Once again, they found that impaired renal function (including inulin clearance, renal plasma flow rate, concentrating ability and ammonium excretion) correlated most closely with tubulointerstitial histological changes.

However, it was the careful and detailed histomorphometric studies of Bohle and colleagues over the last 15 years that put the issue largely beyond doubt. By studying large numbers of renal biopsies of specific diseases including classical "glomerular" diseases, they were able to show that the appearance of the tubulointerstitial compartment in the initial biopsy was the main histological predictor of whether renal function would be preserved or lost [6]. Thus, in extreme cases relatively normal glomerular appearances could co-exist with a severely impaired GFR while highly abnormal glomerular appearances could co-exist with a normal GFR [7]. This apparent anomaly became explicable, however, if concomitant tubulointerstitial changes were also taken into account. Two other relevant factors should also be considered: the age of the patient and the existence of potentially "reversible" increases in interstitial volume. It is well-known that older patients tend to have more interstitial fibrosis in association with decrements in GFR; the cause of this is unknown [8]. Also, interstitial volume may be expanded in inflammation by edema or infiltrating cells without matrix deposition. This expansion could thus resolve spontaneously or in response to treatment, and may provide an explanation for the negative findings of Bennett, Walker and Kincaid-Smith who were unable to find a correlation between the increase in interstitial volume and decline in GFR when serial biopsies of patients with IgA nephropathy were examined [9].

Potential mechanisms of tubulointerstitial injury

How do we reconcile the fact that various diseases which primarily affect glomeruli appear to set in train secondary processes that affect the tubulointerstitium resulting in tubular atrophy, interstitial fibrosis and interstitial mononuclear cell infiltrates? For convenience, our current knowledge can be discussed under several headings: microvascular injury, tubular cell injury, tubular cell-inflammatory cell interactions, altered fibroblastic phenotype and tubular cell-fibroblast "cross-talk."

Microvascular injury

Direct histomorphometry of ultrathin sections of renal biopsies taken from human kidneys with glomerular diseases has demonstrated obliteration of the post-glomerular peritubular capillary network in these kidneys [10]. The functional consequence of this would be tubular cell ischemia [11]. Renal oxygen consumption is already high at rest and may be further increased by adaptive processes in the remnant kidney [12, 13]. Thus, the net result of diminished oxygen delivery and increased oxygen demand would logically be tubular hypoxia and injury.

The mechanisms which lead to a loss of the post-glomerular circulation are not known, but one possibility is systemic

hypertension since hypertension is a common accompaniment of most human renal diseases. It should be noted that tubulointerstitial injury is a prominent histological feature of the remnant kidney [2, 14] but that this finding is absent if blood pressure is not elevated [14]. Several studies both in rats [15] and humans [16] have shown that better control of systemic blood pressure leads to a slowing of the rate of decline of GFR in the diseased kidney. In the remnant kidney, both afferent and efferent arterioles are dilated as part of the adaptive response to increased glomerular blood flow [17]. Thus, it is possible that systemic blood pressure results not only in "glomerular" but also "post-glomerular" hypertension. Certainly, it seems likely that any sustained increase in peritubular capillary pressure could adversely affect endothelial, interstitial and tubular cell function. For example, in a rat remnant kidney model, tubular expression of PDGF in the collecting ducts was increased with poorly controlled blood pressure but was reduced with good blood pressure control [18]. PDGF is not only a potent mitogen for interstitial fibroblasts [19] but is also a very potent vasoconstrictor substance [20]. Thus, the elaboration of various vasoactive substances from endothelial (such as endothelin) [21], interstitial (such as adenosine) [22] and tubular cells (PDGF, angiotensin II, endothelin) [19, 23, 24] may result both in vasoconstriction and increased interstitial fibrosis. This would result ultimately in obliteration of the peritubular capillary network. Bohle has suggested that in the early stages, this rise in post-glomerular resistance could result in glomerular enlargement, but the mechanism for this was not stated [10]. Possibly glomerular capillary distension could lead to hypertrophy of the glomerulus. Since hypertrophic glomeruli are thought more likely to sclerose [25], it seems plausible that the obliteration of the post-glomerular capillary network may be one way in which glomerular hypertrophy and sclerosis may be linked.

The recently demonstrated protective effect of converting enzyme inhibition on the tubulointerstitial component of the fibrosis observed in the remnant kidney may well be mediated by an improvement in the post-glomerular circulation (due to efferent arteriolar dilatation) provided that systemic blood pressure is reduced [18]. If it is not, peritubular injury could well be aggravated.

Tubular cell injury

Hypermetabolism. Renal oxygen consumption is largely determined by the rate of tubular transport, and in many forms of renal disease with hypertrophied tubules this is increased [12]. Any factors that might increase sodium reabsorption, for example high protein diets, will further increase oxygen consumption. Several authors have suggested that increased oxygen consumption by the remnant kidney might itself be damaging. Two possible mechanisms have been proposed: increased ammoniagenesis and increased generation of reactive oxygen species. Increased ammoniagenesis has been linked with increased oxygen consumption in the surviving nephrons of remnant kidneys [26]. The ammonia generated is thought to then activate the third component of complement (C3) which then leads to activation of the alternative complement cascade [27]. This in turn could lead to the influx of inflammatory cells, the elaboration of inflammatory mediators and increased collagen synthesis by tubular cells [28]. C3 could also be synthesized locally by tubular cells and its production upregulated by interleukin 2 secreted locally by T cells [29], thus further fueling the process. Against this hypothesis is the fact that ammonia concentrations increase to very high concentrations normally in the renal medulla without causing injury. However, Clark and his co-workers have shown experimentally that a hyperosmolar milieu is able to protect against injury triggered by increased ammonia concentrations [30].

Increased oxygen consumption may also lead to the increased generation of reactive oxygen species by tubular cells [31, 32]. However, normal anti-oxidant defense mechanisms may be adequate to prevent significant oxidative injury unless a further stress, such as a high protein diet, is imposed on the remnant kidney [32, 33]. Indeed, the dietary depletion of antioxidants imposed on weanling rats is sufficient in itself to lead to proteinuria, a decreased GFR and a tubulointerstitial infiltrate [34]. Reactive oxygen species may also up-regulate collagen gene expression by tubular cells as they do in fibroblasts [35].

Because tubular atrophy is the rule in advanced tubulointerstitial disease, it is reasonable to speculate that the adaptive processes described above occur early, that is, where tubular hypertrophy is observed [36], but that this gives way to atrophy as the tubular cells become damaged by ischemia or toxic molecules.

Filtration of "noxious" molecules. Several studies have shown that proteinuria *per se* is an independent risk factor for the decline in renal function in proteinuric states [37, 38]. Moreover, recent studies suggest that the reduction in proteinuria itself might be beneficial in reducing the rate of decline in GFR [15]. Previously, in two established rat models of glomerulosclerosis, that is, puromycin aminonucleoside and adriamycin treated rats, serial micropuncture studies of the same glomeruli showed that the progressive loss of glomerular function was independent of any change in glomerular capillary pressures which remained normal throughout [39]. Moreover, the beneficial effect of captopril in reducing glomerulosclerosis was independent of its effect on glomerular hemodynamics [39]. It is of interest also that in both these models, tubulointerstitial changes precede the development of glomerulosclerosis [38]. This finding has been extended recently by the observation that enalapril reduces tubulointerstitial damage in the rat remnant kidney model independently of its effect on systemic blood pressure [18]. The exact mechanism for this beneficial effect of angiotensin-converting enzyme inhibitors is unclear, but apart from the protective effect on the postglomerular circulation described above, it could involve an effect in reducing proteinuria.

Apart from albumin, a variety of circulating macromolecules may also be filtered in nephrosis. These include immunoglobulins, haptoglobin, lipoproteins, transferrin and complement (Table 1). In addition, red blood cells are commonly filtered in glomerular diseases.

Several groups have attempted to investigate the potential tubular toxicity of filtered albumin. Eddy developed a model of "protein-overload proteinuria" by injecting bovine albumin into rats. Heavy proteinuria was accompanied by proximal tubular regeneration (neo-expression of vimentin) and an interstitial mononuclear cell infiltrate. Surprisingly, in view of the heterologous albumin used, there was no evidence of immune complex deposition in the glomeruli, tubules or interstitium.

Table 1. Relation of filtered substances to tubulointerstitial injury

Disease model	Nature of tubulointerstitial injury
Overload proteinuria [40]	Increased vimentin expression in proximal tubules due to endocytic protein uptake; tubulointerstitial disease.
Heymann nephritis [41]	Lysozymuria due to failure of normal tubular uptake of filtered lysozyme; proximal tubular injury.
Myeloma (light chain proteinuria) [42]	Light-chain-induced. Impaired transport function in proximal tubule and tubulointerstitial disease.
Puromycin aminonucleoside nephropathy [43]	Evidence of tubulointerstitial damage possibly due to filtered proteins.
Complementuria [44]	Histological damage to proximal tubules(?) secondary to activation of filtered complement proteins by brush border membrane.
Nephrotoxic serum nephritis transferrinuria [45]	Postulated iron release into tubular luman injuries tubular cells (?iron-catalyzed generation of hydroxyl ions); attenuated by iron deficiency.
Erythrocyte constituents [46]	Hem-induced tubular damage possibly mediated by iron-dependent free radical formation.
Hyperlipoproteinemia [47]	Reabsorption of filtered lipoproteins by tubular cells induces tubular injury.

Moreover, T cell depletion did not alter the course of disease, implying that tubulointerstitial injury in this model was not primarily T cell-mediated. The mechanism by which albumin is damaging to proximal tubular cells, however, remains unclear; Eddy speculated that the increased trafficking and degradation of albumin in these cells may lead to the release of degradative enzymes into the interstitium [40].

Taking a different approach, Schreiner has found that the urine of proteinuric rats and humans contains a yet unidentified but specific macrophage lipid chemotactic factor [48]. *In vitro*, cultured rat tubular cells also produce the same factor when exposed to high concentrations of albumin [49]. Thus, in this model filtered albumin may lead to secondary immune injury via the production of chemotactic factors by the "injured" tubule [48].

In spite of the attractiveness of these animal models, albuminuria *per se* cannot be damaging in human disease as no tubulointerstitial injury is seen in minimal change disease in spite of heavy albuminuria. Nevertheless, it is clear that certain pathological proteins such as monoclonal light chains can impair proximal tubular transport and lead to tubulointerstitial injury [42, 50]. Thus, the potential toxicity of other filtered proteins remains to be fully elucidated in relevant disease models.

Tubular cell-immune cell interaction

We have already referred to the possibility that primary tubular injury that is non-immune may result in an interstitial immune infiltrate via the production of tubular-derived chemotactic factors. Several other disease models characterized by primary non-immune tubular injury also point to a different consequence of injury: tubular cells can be induced to present antigen. For example, ischemic acute tubular necrosis in the mouse induces early Class I antigen expression in tubular cells and late Class II expression by tubule and interstitial cells [51]. In another model, a known tubular toxin, mercuric chloride, given at low dose induces early Class II expression by tubular cells followed later by a cell infiltrate and immune glomerular deposits [52]. However, both Class II expression and lymphocytic infiltration were interferon-gamma (or T cell) dependent as shown by blocking experiments with antibody to interferon-gamma [52]. The early pathological significance of Class II MHC expression by tubular cells *in vivo* has been emphasized in both animal [53] and human [54] studies. Early expression of Class II molecules on renal tubular cells prior to the onset of glomerular inflammation and proteinuria could be demonstrated in a mouse lupus model, the MRL-*lpr* model [53]. Such expression was localized to tubules with heavy surrounding mononuclear cell infiltrates [53]. In a few human subjects who underwent serial renal biopsies, Muller was able to demonstrate early aberrant tubular expression of HLA DQ, HLA DP and ICAM-1 prior to the development of an interstitial cell infiltrate and significant fibrosis [54].

Proximal tubular cells can be induced to express Class II antigens *in vivo* [55] and *in vitro* [56] by interferon-gamma. They can also be induced to express ICAM-1 by interferon-gamma, IL-1 and TNF-alpha [57]. In culture, they can function effectively as antigen-presenting cells to MHC-restricted antigen-specific T cells [58]. However, the *in vivo* relevance of these findings is unclear since in a transgenic kidney transplant model, high levels of tubular class II molecule expression could not initiate immune injury within a six month follow-up period [59]. Normal immune interaction between antigen-presenting cells and responder T cells requires the co-expression of both ICAM-1 and Class II molecules, especially if expression of the latter is low [60]. When induced, ICAM-1 has been found on the apical tubular cell surface whereas Class II antigens are located basolaterally [55, 61]. Whether ICAM-1 can be relocated to the basolateral surface in the intact tubule during antigen recognition is not known. If, however, there is prior sublethal tubular cell injury such as with ischemia, rapid redistribution of surface molecules such as ICAM-1 could occur to facilitate tubular cell-T cell interactions [62].

Clearly, a close interaction between tubular cells and immune cells is envisaged. We would argue that a primary glomerular injury which may be immune or non-immune could lead to non-immune tubular cell injury, for example due to ischemia or proteinuria. This could lead to the activation or expansion of the sparse normal resident population of immune cells and promote the influx of new cells initially in response to tubular-derived cytokines such as MCP-1 and IL-8 [63]. Class II expression and ICAM-1 expression would be augmented further through the secretion of lymphokines, thus enabling tubular cells to present antigen and so magnifying the immune process [64]. Neo-antigens in these schemes would be derived from filtered proteins or be "exposed" by ischemia [38, 65].

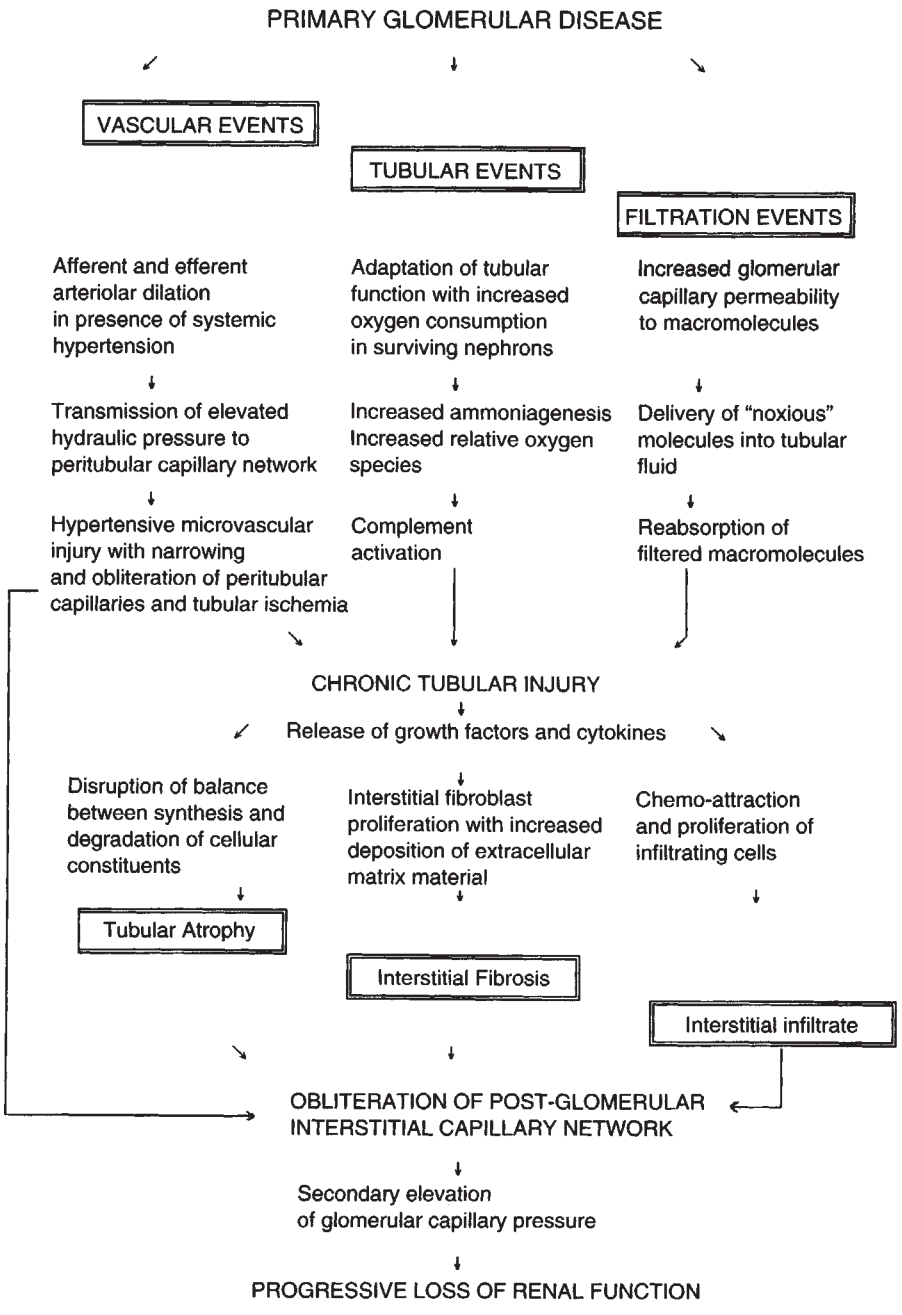

Fig. 1. *Hypothetical sequence of events leading from primary glomerular disease through tubulointerstitial injury to progressive loss of renal function.* (Reproduced by permission of *Eur J Clin Invest* [79]).

Fibroblasts are not the only source of interstitial collagens. Although tubular cells normally synthesize collagen types IV and V (basement membrane), under certain conditions they may also secrete interstitial collagens: types I and III. In a mouse model of autoimmune interstitial nephritis, a T cell-derived tubular antigen-binding protein could be shown to specifically down-regulate transcription of type IV collagen by tubular cells [66]. Simultaneously, type I collagen synthesis appeared to be up-regulated *in vivo* by other factors [66]. This study illustrates the close interaction between tubular cells and immune cells. Just as tubular derived factors may modulate immune cell function, factors derived from immune cells may modulate tubule function [67].

An altered fibroblast phenotype

Several groups have now demonstrated that fibroblasts taken from sites of inflammation and fibrosis from several organs appear to show a stable altered phenotype *in vitro* [68]. In the kidney, Rodemann and Muller have shown in a limited number of cases that human fibroblasts taken from fibrotic kidneys (FKF cells) are hyperproliferative and produce more collagen than normal renal fibroblasts (NKF) [69]. FKF cells also produce a novel protein, yet to be fully characterized, which has been named "fibrosin." By clonal culture analysis, they have also shown that FKF cells are comprised of more "mitotic" than "post-mitotic" cells, implying activation of a putative

fibroblast stem cell population [69]. Intriguingly, they reported that FKF-conditioned medium could make normal human kidney and skin fibroblasts hyperproliferative [69]. The latter observation suggests that FKF cells may be involved in an autocrine loop where an initial stimulus has switched on autocrine production of a growth factor which then continues to stimulate its own production by positive feedback. Examples where this has been shown include platelet-derived growth factor (PDGF) in mesangial cells [70] and acidic-fibroblast growth factor (a-FGF) in polycystic human kidney fibroblasts [71]. An alternative explanation for the stable alteration in phenotype is a somatic mutation. There is evidence that smooth muscle cells derived from human atherosclerotic plaques show hyperproliferative activity, enhanced *myc* proto-oncogene expression and transforming activity [72]. Clearly, a somatic mutation could account for this without the need to invoke gross chromosomal changes or malignant transformation. For the time being, however, the exact mechanism for the observed alteration in phenotype, whether genetic or epigenetic, must remain an open question. Nevertheless, the induction of a stable hyperproliferative phenotype in renal interstitial fibroblasts taken from fibrotic kidneys goes some way to explain the progressive nature of renal disease.

Tubular cell-fibroblast "cross-talk"

Increasingly, tubular cells have been found to be a rich source of a variety of molecules including chemotactic cytokines such as MCP-1 and IL-8 [63], multifunctional pleiotropic cytokines such as IL-6 [73] and TNF-α [56], complement components such as C3 [29], growth factors such as PDGF, IGF-1, TGF-β, EGF, GM-CSF [73–75] and vasoactive peptides such as endothelin, angiotensin II [23, 24, 76]. Although the physiological role of most or all of these factors in the normal kidney is unknown, it can be assumed that tubular cell "dysfunction," for example secondary to proteinuria, may lead to dysregulation of the synthesis and secretion of these factors, further contributing to tubulointerstitial injury by their actions on interstitial fibroblasts and immune cells. One such paracrine loop was demonstrated by *in vitro* studies of rabbit kidney [19, 77]. In culture, inner medullary collecting duct (IMCD) cells secreted a variety of growth factors including PDGF, IGF-1 and TGF-β. However, when IMCD cells and papillary fibroblasts were co-cultured, papillary fibroblast proliferation could be blocked only by antibodies to PDGF but not IGF-1. Furthermore, the absence of an identical loop in the cortex suggested that this loop was specific to the inner medulla. It remains highly likely that other similar paracrine loops operate at different segments of the nephron but remain to be discovered. Interestingly, increased PDGF-receptor sites on interstitital fibroblasts have been demonstrated in transplanted human kidneys undergoing chronic rejection [78], implying a role for PDGF in the tubulointerstitial pathology of this disease.

Tubulointerstitial injury and loss of glomerular function: A possible sequence of events

In this review, we have attempted to show that there is now sufficient experimental evidence to support our contention that an acute primary glomerular insult may set into train secondary chronic tubulointerstitial disease processes which gradually lead to the loss of glomerular function. Figure 1 summarises a possible sequence of events according to the evidence so far. The complexity of the pathways envisaged and our relative ignorance of their finer points imply that only a limited understanding of these pathological processes is possible at this stage. For the moment, however, the seemingly paradoxical loss of GFR which correlates with tubulointerstitial damage is becoming easier to explain.

Acknowledgments

We acknowledge the contribution of Mrs. Berti Rooke-Ley and Miss Judith Hapgood in the preparation of this manuscript.

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